

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. : 10/541,246 Confirmation No.: 5820

Applicant(s) : Mark L. Lawrence

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Title : Methods of preparation of live attenuated bacterial vaccine by alteration of dna adenine methylase (*dam*) activity in those bacteria

TC/A.U. : 1645

Examiner : Navarro, Albert Mark

Docket No. : 028186.61646

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner:

I, Mark L. Lawrence, do hereby declare the following:

- (1) I am a named inventor of the subject matter of the above-identified patent application.
- (2) I am an Associate Professor at the College of Veterinary Medicine at Mississippi State University with my primary areas of expertise being bacterial pathogenesis, molecular microbiology, and aquatic animal health. I received my Bachelor of Science degree in 1988 from Texas A&M University, a Doctorate of Veterinary Medicine from Texas A&M University in 1990, a Ph.D. in 1997 from Louisiana State University, and performed Post Doctoral work at Virginia Tech.
- (3) It cannot be assumed that the DNA adenine methylase (*dam*) gene would be present in *P. multocida*.
- (4) Several bacterial species exist that do not have the *dam* gene, including gram-negative, and pathogenic bacteria. Table 1 provides examples of bacterial species from the Comprehensive Microbial Resource database that have fully sequenced

genomes, but do not have a DNA adenine methylase gene annotated in their genome. The website showing information about the genome is included in the column on the right.

Table 1

Species	Website showing Genome Page
Acinetobacter radioresistens	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=hmp082
Agrobacterium tumefaciens	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntat01
Anaplasma marginale	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntam01
Arcobacter butzleri	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntab14
Bordetella bronchiseptica	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntbb01
Bordetella parapertussis	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntbp02
Bordetella pertussis	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntbp03
Bartonella henselae	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntbh02
Brucella abortus	
Brucella melitensis	
Brucella suis	
Buchnera aphidicola	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntba03
Caulobacter crescentus	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=gcc
Coxiella burnetii	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=gcb02
Ehrlichia canis	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntec07
Enterobacter sp. 638	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntes04
Francisella tularensis	
Listeria monocytogenes	
Mannheimia succiniciproducens	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntms01
Mycobacterium tuberculosis	
Neorickettsia sennetsu	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ges
Nitrobacter hamburgensis	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntnh01
Oligotropha carboxidovorans	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntoc02
Pseudomonas aeruginosa	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntpa03
Ralstonia solanacearum	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntsr01
Rhodopseudomonas palustris	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntpr04
Staphylococcus aureus	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntsa44
Wolbachia pipientis	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=gbw
Xanthomonas campestris 8004	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntxc02
Zymomonas mobilis ZM4	http://cmr.jcvi.org/cgi-bin/CMR/GenomePage.cgi?org=ntzm01

- (5) *Mannheimia succiniciproducens* is in the same family as *Pasturella multocida*, but it does not have a *dam* gene present in its genome.

- (6) Based on the vast amount of bacteria without *dam* genes, including some within the same family as *P. multocida*, it would not be obvious to a person of ordinary skill in the art that the *dam* gene would be present in *P. multocida*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Mark L. Lawrence

Date: 5/28/10